

Serum titres of *Pneumocystis carinii* antibody in health care workers caring for patients with AIDS

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Abstract

Background—*Pneumocystis carinii* pneumonia was thought to occur from reactivation of latent infection, but recent studies with the polymerase chain reaction have failed to detect *P carinii* in normal subjects. If pneumocystis pneumonia is therefore caused by new infection the source and mode of transmission of *P carinii* remains unknown.

Methods—Natural exposure to *P carinii* was detected by measuring antibodies by indirect immunofluorescence in 24 health care workers working continuously with patients with AIDS and 24 control health care workers exclusively treating elderly patients.

Results—*P carinii* antibody titres were significantly higher in the health care workers exposed to AIDS than in the control group (median titre 1:32 *v* 1:16 respectively). Three control subjects had no antibodies compared with none of the subjects exposed to AIDS, and 10 of the 12 highest titres came from the exposed group.

Conclusions—Raised *P carinii* antibody titres in health care workers caring for patients with AIDS suggest that patients infected with HIV may be a potentially infectious source of *P carinii* for susceptible subjects.

(Thorax 1993;48:619-621)

reaction.^{9,10} Pneumocystis pneumonia may therefore develop from a new infection with *P carinii* rather than from latent infection. This has raised questions about possible sources of *P carinii* and, with recent case reports of pneumocystis pneumonia occurring after documented exposure,^{11,12} suggests that subjects with active pneumocystis pneumonia may be potentially infectious. Although airborne *P carinii* causes pneumonia in rats,¹³ the same has not been shown to occur in humans. If, like rats, humans become infected with *P carinii* by this route high antibody titres would be expected in immunocompetent subjects working closely with patients with AIDS, who are known carriers of the organism.¹⁴ We therefore compared *P carinii* antibody titres in health care workers working with patients with AIDS and in a control group of health care workers.

Methods

Two groups of 24 health care workers were studied; all subjects lived and worked in London. The first group (17 women, seven men, mean age 33 years) had worked exclusively with patients with AIDS or those seropositive for HIV for at least six months before the study (mean duration 3.2 years) in a unit where about 200 new cases of pneumocystis pneumonia are diagnosed annually. The second, control, group (23 women, one man, mean age 42 years) had worked exclusively in dedicated geriatric hospitals and had never knowingly cared for patients positive for HIV or worked in a hospital treating patients with AIDS. All subjects were healthy and receiving no immunosuppressive drugs. Apart from two homosexuals in the exposed group who were negative for HIV in serial tests, all subjects were at low risk of HIV infection. Specimens of serum were taken from all subjects and saved at -80°C for antibody testing.

DETECTION OF ANTIBODIES

All specimens were randomised and coded to ensure blind testing for antibodies. *P carinii* antibodies were detected by indirect immunofluorescence using sections of human postmortem lung which were heavily infected with *P carinii*. These sections had been fixed in Bouin's fluid to destroy existing immunoglobulin and preserve the pneumo-

In patients with AIDS *Pneumocystis carinii* pneumonia still has a mortality of between 12% and 33%,^{1,2} but a full understanding of its natural history and mode of transmission has been hindered by a failure to culture the organism in vitro. Serological studies have shown early acquisition of *P carinii* antibodies,³ with a seroprevalence of between 8.8% and 90% in normal adults.⁴⁻⁷ Such findings led to the widely accepted hypothesis that most people have subclinical latent infection with *P carinii* and that pneumocystis pneumonia results from reactivation of this latent infection after immunosuppression.⁸ This reactivation hypothesis has recently been questioned by several studies that have failed to detect latent *P carinii* infection in normal subjects despite the use of highly sensitive techniques using the polymerase chain

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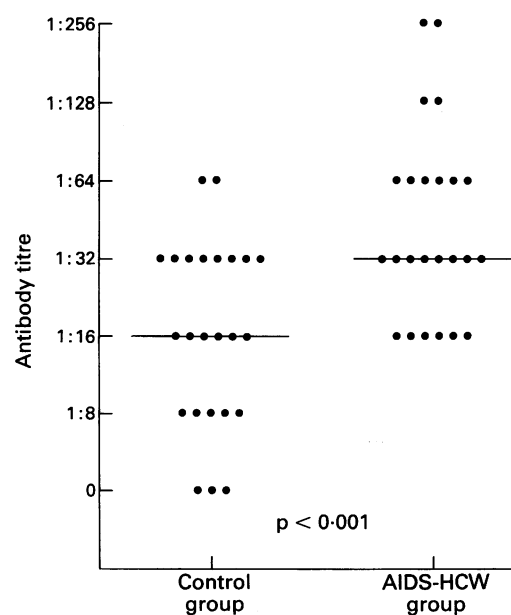
Received 6 November 1992
Returned to authors
26 January 1993
Revised version received
10 February 1993
Accepted 16 February 1993

cystis polysaccharide antigen. Sections were deparaffinised, rehydrated, and washed for 30 minutes in phosphate buffered saline at pH 7.4. The slides were then reacted for 30 minutes with a sample of each subject's serum diluted 1:8 with phosphate buffered saline. After washing, the slides were treated for 30 minutes with a 1:32 dilution of sheep derived fluorescein labelled whole anti-human immunoglobulin (Murex Diagnostics, Dartford). After washing, slides were mounted under phosphate buffered saline/glycerol (Citifluor, Citifluor Ltd, London) to preserve fluorescence, and viewed at 50× magnification under oil immersion and ultra violet light. Positive and negative control serum samples were included to confirm adequate staining, and all slides were viewed independently by two observers. Positive specimens, identified by clumps of fluorescing pneumocysts, were tested further at increasing, doubling, serum dilutions—that is, 1:16, 1:32, etc—until a negative result was obtained, and specimens giving different results between the two observers were repeated. Specimens with titres of 1:128 or more were also tested for IgM specific *P. carinii* antibody with the same method but substituting IgM specific anti-human antibody for the whole antihuman immunoglobulin (Murex Diagnostic, Dartford). Results for the two groups were analysed, after breaking the code, with the Mann-Whitney test.

Results

There was good overall agreement between the two observers, with a concordance rate of 90%. A high background fluorescence, the most frequent cause of discrepancies, was invariably resolved after restaining.

Overall, 45 of the 48 specimens (94%) had



P. carinii total antibody titres with median values in health care workers caring for patients with AIDS (AIDS-HCW) and those caring for elderly patients (control group).

P. carinii IgM titres in four patients with total antibody titres of 1:128 and above

| Case no | Total antibody titre | IgM titre |
|---------|----------------------|-----------|
| 1 | 1:256 | 1:8 |
| 2 | 1:256 | 1:64 |
| 3 | 1:128 | — |
| 4 | 1:128 | — |

detectable antibody to *P. carinii* at dilutions of 1:8 and above (fig). The three specimens negative for antibody all came from the control group, whereas the four samples with titres of 1:128 and above (which were also tested for *P. carinii* IgM) came from the group exposed to AIDS. Antibody titres in the exposed group were significantly higher than in the control group, with a median titre of 1:32 (range 1:16 to 1:256) v 1:16 (0 to 1:64), respectively ($p < 0.001$). IgM was not detectable in samples from the two subjects with total antibody titres of 1:128, and IgM titres of 1:8 and 1:64 were found in the samples from the other two subjects with total antibody titres of 1:256 (table). Neither of these latter two subjects had recently had respiratory illness and both remained symptom free. One (case 1) was a nurse responsible for helping with inpatient bronchoscopies to investigate patients with suspected pneumocystis pneumonia, and the other (case 2) was a research nurse who had been working with patients with AIDS for seven years. We found no relation between antibody titre and length of time working with patients infected with HIV or between the antibody titres in medical and nursing staff.

Discussion

The finding of an overall high prevalence of *P. carinii* antibodies agrees with most other seroprevalence studies. The wide variation in the prevalence detected in these studies, ranging from 8.8% of blood donors,⁴ 57% of normal controls,⁵ and 85% of normal adults,^{6,7} to 100% of two year old children,¹⁵ may be partly explained by different laboratory techniques but may also reflect geographical or seasonal differences in the prevalence of *P. carinii*. Although animal studies have clearly shown the infectious nature of *P. carinii*,¹³ recommendations to isolate infected individuals¹⁶ have been largely ignored in the belief that the population is already latently infected with *P. carinii*. With mounting evidence refuting this hypothesis of latent infection,^{9,10} the potentially infectious nature of *P. carinii* has become more important.

Our results support the hypothesis that *P. carinii* may be potentially infectious by showing raised antibody titres and therefore exposure to *P. carinii* in health care workers caring for patients with AIDS compared with control health care workers. Although we could not completely exclude the possibility of covert infection with HIV without resorting to serial HIV tests, our selection criteria kept this possibility to a minimum. Differences in the age and sex distribution of

the two groups were small and probably did not skew the difference in antibody titres between the two groups. Another possible source of error was the two homosexuals in the exposed group, who may have been exposed to *P carinii* socially rather than professionally. Both their antibody titres, however, were below the group average at 1:16, and they had no haematological evidence of immune paresis.

The presence of significant IgM titres in association with the highest total antibody titres may indicate that these individuals had been recently exposed to *P carinii*, although both subjects remained free of respiratory symptoms. The high antibody titres of the bronchoscopy nurse are noteworthy because she was probably being regularly exposed to fluid aerosols containing *P carinii*. Although we cannot eliminate other, unidentified, sources of *P carinii* as the cause of raised antibody titres, these findings suggest that patients with AIDS may be a potentially infectious source of *P carinii* to susceptible individuals. As different serotypes of human *P carinii* can now be distinguished by molecular biological techniques, some of the questions about the epidemiology and transmission of *P carinii* infection may soon be resolved.

In conclusion, this study adds to the growing evidence that infection with *P carinii* may be transmitted from infected carriers, who may be a potential source of pneumocystis pneumonia in immunosuppressed subjects.

We thank Dr David Warhurst of the London School of Hygiene and Tropical Medicine for supplying the fixed blocks of human postmortem lung. TRL is supported by a grant from North West Thames Regional Health Authority.

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